REMARKS

Claims 10 and 44 have been canceled. Claims 1-6, 9, 12, 15, 16, 19-22, 25-39, 43, 46, 47, 49, 52, 55 and 58-60 have been amended to obviate the indefiniteness rejection as specified below with respect to this rejection. It is submitted that these amendments do not constitute new matter and their entry is requested.

The Examiner has rejected claims 63-81 under 35 USC §112, first paragraph for lack of written description. The Examiner contends that Applicants have not provided a written description of the cultures and plants produced by the claimed method. It is submitted that the Examiner is in error in this rejection.

Applicants have invented a method which provides for enhanced transformation and regeneration of transformed embyrogenic pine tissue in which the pine is of the genus *Pinus*, subgenus *Pinus*, as well as various aspects of this method. The method involves minimizing damage to cells subsequent to *Agrobacterium* infection and rapidly selecting transformed cells. The transformed cells are cultured to produce transgenic somatic embyros which are then germinated to produce transgenic plants. Applicants discovered that, through the use of the disclosed and claimed method and its various aspects, they were able to transform and regenerate transgenic pine plants of the *Pinus* subgenus, i.e. hard pines. Applicants' invention allowed for the first time the transfomation and regeneration of transgenic plants of pine of the subgenus *Pinus*, i.e., hard pines, especially at significant frequency. The present application contains a written description of this method.

According to the Written Description Guidelines, a patent specification must describe the invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. Possession of the claimed invention is shown by describing the claimed invention with all of its limitations using such descriptive means as **words**, structures, figures, diagrams, and formulas that fully set forth the claimed invention (emphasis added). Possession may be shown in a variety of ways including description of an actual reduction to practice. An adequate written description may be shown by any description of sufficient, relevant

identifying characteristics so long as a person skiled in the art would recognize that the inventor had possession of the claimed invention.

In the present application, Applicants have clearly conveyed to a skilled artisan what was intended and what was invented. Applicants described **by words** a method which provides for enhanced transformation and regeneration of transformed embyrogenic pine tissue in which the pine is of the genus *Pinus*, subgenus *Pinus*, as well as various aspects of this method. The method, as described, produces a transgenic embyrogenic pine culture, and upon regeneration, a transformed pine plant for the *Pinus* subgenus. Applicants further described an actual reduction to practice of the claimed invention. The specification includes a description of an actual reduction to practice not only of the method, but also of the tissue culture produced by the method and transformed plants produced by the method. Thus, a skilled artisan can readily recognize that Applicants were in possession of the claimed invention.

Furthermore, the specification describes products produced by the process. These products are described on the basis of product-by-process language. Product-by-process language has been approved by the Patent Office and the courts, including the Federal Circuit. The *Eli Lilly* decision cited by the Examiner did not exclude product-by-process as a means of claiming subject matter. The specification clearly conveys to a skilled artisan what was invented by describing the method and by showing products (i.e., tissue culture and transformed plants) produced by the process. Thus, a skilled artisan can readily recognize that Applicants were in possession of the claimed invention.

Finally, the Examiner attempts to apply the *Eli Lilly* decision to the facts of the present case by arguing that Applicants failed to describe the genetic material, and as a result the specification fails to provide a written description of the claimed invention. However, *Eli Lilly* was concerned with DNA having only a functional definition. The present case is not directed to DNA, but rather to a method which provides for enhanced transformation and regeneration of transformed embyrogenic pine tissue in which the pine is of the genus *Pinus*, subgenus *Pinus*, and to products of this method, namely a tissue culture (which is an intermediate product) and a transformed plant (the final product). Since Applicants are not claiming any DNA, a description of such is not required

by the patent statutes or the case law. Thus, a skilled artisan can readily recognize that Applicants were in possession of the claimed invention.

In view of the above reasons, it is submitted that the specification contains an adequate written description of the claimed invention, i.e., products by process. Withdrawal of this rejection is requested.

The Examiner has rejected claims 1-9, 12-16, 20, 22, 25-43, 46-48, 52-54, 58, 60 and 63-81 under 35 USC §102(b) as being anticipated by Levee et al. The Examiner contends that Levee et al. teaches a method for the stable transformation of *Pinus strobes* after cocultivation of embryogenic tissues with *Agrobacterium* in which a support membrane is used. It is submitted that Levee et al. does not disclose the elements of the claimed invention and hence cannot anticipate the claimed invention.

Specifically, Levee et al. discloses *Agrobacterium* transformation of white pine, *Pinus strobes*. As is well known in the art, white pine is a soft pine and not a hard pine. As is evident in the name, *Pinus strobes*, white pine is a member of the subgenus *Strobes* and is not a member of the subgenus *Pinus*. For example, most classifications of *Pinus* recognize two major lineages: subgenus *Strobus* (haploxylon or soft pines, with one fibrovascular bundle in the needle) and subgenus *Pinus* (diploxylon or hard pines, with two fibrovascular bundles in the needle). This division is consistent with data from wood anatomy and secondary chemistry, and is supported in recent molecular phylogenetic studies (Strauss and Doerksen, 1990, *Evolution* **44**:1081-1096; Wang and Szmidt, 1993, *Plant Systematics and Evolution* **188**:197-211; reviewed in Price et al., 1998, in *Ecology and Biogeography of Pinus*, Cambridge University Press, Cambridge, pp. 49-68).

Pines have a relatively rich fossil record dating back to the Early Cretaceous, 130 million years ago (review in Axelrod et al., 1986, *Ann Mo Bot Gard* **73**:565-641; Klaus et al., 1989, *Plant Systematics and Evolution* **162**:133-163; Van der Burgh, 1973, *Review of Paleobotany and Palynology*, **15**:73-275; Millar, 1993, *Ann Mo Bot Gard* **80**:471-498). The genetic distance between subgenera, at least between *Pinus* and *Strobus*, may be as large as, or larger than the genetic distance between other conifer genera, e.g., between *Cedrus* and *Abies* (Price et al., 1987, *Systematic Botany*, **12**:91-97), and if strict genetic criteria were used, they should perhaps be treated at generic rank. As

is commonly known, hard pines are unable to breed with soft pines, though they can interbreed readily, if the correct timing and other conditions are provided, with other hard pine species (a seminal reference is Critchfield and Little, 1966, *Geographic distribution of the pines of the world*, USDA Forest Service Miscellaneous Publication 991, Washington, D.C.; see also Little and Critchfield, 1969, *Subdivision of the genus Pinus pines*, USDA Forest Service Miscellaneous Publication 1144, Washington, D.C.). Hard pines are unaffected by a number of diseases, such as white pine blister rust, that readily infect soft pines. Their susceptibility to *Agrobacterium* infection appears to be quite different as well.

Levee et al. discloses the transformation and regeneration of pine of the subgenus *Strobus* which, according to this reference, "is the first work on genetic transformation on **this pine species** as well as the first report of successful stable genetic transformation of **a pine species** using a disarmed strain of *A. tumefaciens*". (See page 36, first paragraph of Discussion, emphasis added). Levee et al. does not disclose the transformation and regeneration of pine of the subgenus *Pinus*. The amended claims are clearly directed to pine cells of the *Pinus* subgenus. It is well known to those skilled in the art that somatic embyrogenesis systems for soft pines are different from those for hard pines. It is not insignificant that Levee et al. utilized a soft pine which is more easily regenerated than hard pines. Although the Examiner cited art showing transformation and regeneration of soft pine, he has not cited any art showing transformation and regeneration of hard pines as set forth in the claims. Furthermore, it is submitted that there has been no reports in the literature of the regeneration of plants following stable transformation of embryogenic cultures of any pines of the *Pinus* subgengus by *Agrobacterium*. Since Levee et al. does not teach pine cells of the *Pinus* subgenus, it cannot anticipate the claimed invention. For these reasons, it is submitted that Levee et al. does not anticipate the claimed subject matter, and withdrawal of this rejection is requested.

The Examiner has rejected claims 1-81 under 35 USC §103(a) as being obvious over Levee et al. The Examiner contends that Levee et al. teaches a method for the stable transformation of *Pinus strobes* after cocultivation of embryogenic tissues with *Agrobacterium* in which a support membrane is used. Since Levee et al. does not disclose regeneration of stably transformed

embryogenic cultures of pines of the *Pinus* subgenus and in view of the known differences between soft pines (Levee et al.) and hard pines (the present invention) as discussed above, it is submitted that Levee et al. does not establish a *prima facie* case of obviousness of the claimed invention specifically directed to hard pines, i.e., pines of the *Pinus* subgenus. Therefore, the cited reference cannot not render the claims obvious. Withdrawal of this rejection is requested.

The Examiner has rejected claims 1-81 under 35 USC §112, second paragraph, for being indefinite. Applicants initially note that claims are not read with blinders or based on their literal language but are read in view of the knowledge possessed by one skilled in the art. The words of the claims are read in their context, not in isolation, and as a skilled artisan would read them. In this context, the claims and the Examiner's rejection are discussed.

Claims 1, 2, 5, 6, 20, 25, 27-30, 32-38 and 52 have been amended to change the language "subjecting" to "incubating," although the language ("subjecting") is well known to a skilled artisan and conventionally used in patents. It is submitted that use of the term "incubating" is definite.

Claims 1 and 25 have been amended to indicate that the damage to the cells is physical damage or loss of cells (see page 8, line 26 - page 10, line 10) and the minimized damage is assessed by reference to pre-transformation growth rates (see page 8, line 34). This amendment carries through to the dependent claims, such as claims 2, 4, 26, 31 and 39. It is submitted that this amendment defines the type of damage and how it is determined, thereby rendering the claims definite.

Claims 1, 12 and 25 have been amended to delete the language "rapidly." As set forth in the claims, transformed cells are selected. Any means known to skilled artisans which selects transformed cells can be used. Thus, the claims are definite.

It is submitted that the use of the term "germinating" in claims 1 and 25 is not indefinite. It is well known in the plant regeneration art that somatic embryos are germinated to produce plantlets. Embryos, zygotic (seeds) and somatic (tissue culture), are each germinated to produce plants. The fact that this language is art-recognized is demonstrated in Levee et al., cited by the Examiner. Levee et al. indicates that plantlets are germinated from somatic embryos. Thus, it is submitted that the use of the term "germinating" in claims 1 and 25 is definite.

Claims 2, 4 and 25 are definite in view of the previously described amendment to claims 1 and 25 concerning the term "damage".

Claims 2, 26 and 39 have been amended to change the language "resuspending" to "suspending." It is submitted that this term is definite.

Claims 2 and 26 have been amended to make clear that it is the liquid wash medium containing the suspended cells which is agitated. It is submitted that a skilled artisan readily knows how to agitate liquids, and hence the claims are definite.

Claims 2, 4, 26 and 31 have been amended to insert the language "and."

Claims 2, 4, 31 and 39 have been amended to specify that washed cells are recovered. These claims are definite in view of the previously described amendment to claims 1 and 25 concerning the term "minimized damage". Since the term "recovering" is conventionally used in the art (see, for example, U.S. Patent Nos. 6,143,562 and 5,821,126), it is submitted that this term is known by a skilled artisan and hence definite, as are claims 2, 4, 31 and 39.

With respect to the language "support membrane" in claims 3, 5, 6, 11, 12, 14, 18, 24, 27, 29, 30, 32-37, 39, 40, 45-48, 51, 52, 54, 57, 58 and 62, Applicants note that the claims specify that the cells are plated onto the support membrane. Thus, it is submitted that it is clear from the language of the claims what is supported, and that this language is therefore definite.

Claims 2, 4, 26 and 31 have been to amended to indicate that the cells are rinsed to remove *Agrobacterium*.

Since the term "recovering" is conventionally used in the art (see, for example, U.S. Patent Nos. 6,143,562 and 5,821,126), it is submitted that this term is known by a skilled artisan and hence definite, as are claims 2, 4, 20, 26, 29, 30, 31, 35, 36, 38, 39 and 58.

Although *Agrobacterium* is used for the transformation of pine cells (in the present invention) by incubating pine cells with the *Agrobacterium*, once the incubation is completed, the *Agrobacterium* is then a contaminant and must be removed, as is well known in the art, including the art cited by the Examiner (Levee et al.). As is well known in the art, contamination is no longer detectable when cultures of the pine cells subjected to *Agrobacterium* infection no longer show *Agrobacterium* growth. Since skilled artisans readily know that contaminating *Agrobacterium* must

be removed and know how to detect *Agrobacterium* contamination, it is submitted that claims 7 and 41 are definite.

Claims 9 and 43 have been amended to incorporate the language of claims 10 and 44, respectively. It is submitted that this amendment renders these claims definite.

Claims 9 and 43 have been amended to delete the language relating to "interfering."

Claims 12 and 25 have been amended to delete the language "rapid."

Claims 19 and 25 have been amended to that the *Agrobacterium* has been eradicated from the pine cells.

Claims 21, 22, 49, 59 and 60 have been amended to delete the language "thin."

Claims 21, 22, 49, 59 and 60 have been amended to change the language "film" to "layer."

Claim 29 depends on claim 26 which depends on claim 25. Claim 38 depends on claim 25. Claim 25 has one step directed eradicating *Agrobacterium*. Thus, there is proper antecedent basis in claims 29 and 38 for "said eradication."

Since the term "recovering" is conventionally used in the art (see, for example, U.S. Patent Nos. 6,143,562 and 5,821,126), it is submitted that this term is known by a skilled artisan and hence definite, as are claims 29-31, 35, 36, 38, 39 and 58.

Claim 39 is definite in view of the previously described amendment to claim 25 concerning the term "minimized damage".

It is submitted that claim 41 is definite with respect to the term "contamination" as discussed above.

Claim 47 has been amended to change "culture components" to "tissue culture constituents." Support for this language can be found at page 10, line 16.

It is believed that these amendments and remarks obviate the indefiniteness rejection. Withdrawal of this rejection is requested.

Finally, Applicants note that an Information Disclosure Statement (IDS) was filed on 23 May 2002. Since this IDS was filed subsequent to the mailing date of this Office Action and since a Supplemental IDS is being filed concurrently herewith, Applicants are submitting the requisite fee

to have these papers considered by the Examiner. It would be appreciated if the Examiner would return copies of the art citations that have been initialed and dated with the next communication.

In view of the above amendments and remarks, it is believed that the claims satisfy the requirements of the patent statutes and are patentable over the cited prior art. Reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

<table>
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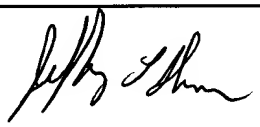
<tr><td>Signature</td><td colspan="2">  </td><td>Date</td><td colspan="2">15 November 2002</td></tr>
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Attachments: Marked-Up Copies of Amendments

Marked-up Copy of Amended Claims

1 (amended). A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

incubating ~~subjecting~~ pine cells of the *Pinus* subgenus with ~~to~~ *Agrobacterium* ~~infection~~ for *Agrobacterium* transformation;

minimizing damage to cells subsequent to *Agrobacterium* infection, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

~~rapidly~~ selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

2 (amended). The method of claim 1, wherein said damage to cells is minimized by:

(a) suspending ~~resuspending~~ cells having been incubated with *Agrobacterium* ~~subjected to transformation~~ in a liquid wash medium;

(b) agitating ~~said cells in~~ said liquid wash medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed~~, transformed~~ cells with minimal damage.

3 (amended). The method of claim 2, wherein pine cells are plated onto a support membrane prior to ~~subjecting to~~ *Agrobacterium* transformation.

4 (amended). The method of claim 1, wherein said damage to cells is minimized by:

(a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;

(b) rinsing said cells using a liquid wash medium to remove *Agrobacterium*; and

(c) recovering washed~~, transformed~~ cells with minimal damage.

5 (amended). The method of claim 4, wherein pine cells are plated onto a support membrane prior to ~~subjecting to~~ *Agrobacterium* transformation.

6 (amended). The method of claim 4, wherein pine cells are plated onto a support membrane subsequent to ~~subjecting to~~ *Agrobacterium* transformation.

9 (amended. The method of claim 4, wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium ~~without interfering with subsequent growth of the plant cells~~, said wash carried out for between half an hour to overnight in duration.

12 (amended). The method of claim 1, wherein said ~~rapid~~ selection is performed by

culturing cells which have been incubated with *Agrobacterium* ~~subjected to transformation~~ on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and

selecting transformed cells.

15 (amended). The method of claim 14, wherein said layer is a ~~thin film~~ layer of liquid medium.

16 (amended). The method of claim 14, wherein said layer is a ~~thin film~~ layer of gelled medium.

19 (amended). The method of claim 1 which further comprises ~~the~~ eradication of *Agrobacterium* from the pine cells after incubation with *Agrobacterium*.

20 (amended). The method of claim 19, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* ~~subjected to transformation~~ on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and

recovering cells from which said *Agrobacterium* has been eradicated.

21 (amended). The method of claim 20, wherein said layer is a ~~thin film~~ layer of liquid medium.

22 (amended). The method of claim 20, wherein said layer is a ~~thin film~~ layer of gelled medium.

25 (amended). A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

incubating ~~subjecting~~ pine cells of the subgenus *Pinus* with ~~to~~ *Agrobacterium* ~~infection~~ for *Agrobacterium* transformation;

eradicating *Agrobacterium* from the pine cells after incubation with *Agrobacterium*;

minimizing damage to cells subsequent to *Agrobacterium* infection, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

~~rapidly~~ selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

26 (amended). The method of claim 25, wherein said damage to cells is minimized by:

(a) suspending ~~resuspending~~ cells having been incubated with *Agrobacterium* ~~subjected to transformation~~ in a liquid wash medium;

(b) agitating ~~said cells in~~ said liquid wash medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed~~, transformed~~ cells with minimal damage.

27 (amended). The method of claim 26, wherein pine cells are plated onto a support membrane prior to ~~subjecting to~~ *Agrobacterium* transformation.

28 (amended). The method of claim 26, wherein said ~~rapid~~ selection is performed by

culturing cells which have been incubated with *Agrobacterium* ~~subjected to transformation~~ on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and

selecting transformed cells.

29 (amended). The method of claim 26, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* ~~subjected to transformation~~ on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and

recovering cells from which said *Agrobacterium* has been eradicated.

30 (amended). The method of claim 28, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* ~~subjected to transformation~~ on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and

recovering cells from which said *Agrobacterium* has been eradicated.

31 (amended). The method of claim 25, wherein said damage to cells is minimized by:

(a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;

(b) rinsing said cells using a liquid wash medium to remove *Agrobacterium*; and

(c) recovering washed~~, transformed~~ cells with minimal damage.

32 (amended). The method of claim 31, wherein pine cells are plated onto a support membrane prior to ~~subjecting to~~ *Agrobacterium* transformation.

33 (amended). The method of claim 31, wherein pine cells are plated onto a support membrane subsequent ~~to subjecting~~ to *Agrobacterium* transformation.

34 (amended). The method of claim 31, wherein said ~~rapid~~ selection is performed by

culturing cells which have been incubated with *Agrobacterium* ~~subjected to transformation~~ on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and

selecting transformed cells.

35 (amended). The method of claim 31, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* ~~subjected to transformation~~ on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and

recovering cells from which said *Agrobacterium* has been eradicated.

36 (amended). The method of claim 34, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* ~~subjected to transformation~~ on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and

recovering cells from which said *Agrobacterium* has been eradicated.

37 (amended). The method of claim 25, wherein said ~~rapid~~ selection is performed by

culturing cells which have been incubated with *Agrobacterium* ~~subjected to transformation~~ on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and

selecting transformed cells.

38 (amended). The method of claim 25, wherein said eradication is performed by:

culturing cells which have been incubated with *Agrobacterium* ~~subjected to transformation~~ on a support membrane over a layer containing an eradicant, said layer in or positioned over a gel medium; and

recovering cells from which said *Agrobacterium* has been eradicated.

39 (amended). A method for minimizing damage to transformed cells of pine of the genus *Pinus* subgenus *Pinus* following infection by *Agrobacterium* for *Agrobacterium* transformation which comprises:

(a) washing transformed cells of the subgenus *Pinus* in a liquid wash medium;

(b) plating said cells on a support membrane;

(c) suspending ~~resuspending~~ said cells in a liquid wash medium; and

(d) recovering washed~~, transformed~~ cells with minimal physical damage.

43 (amended). The method of claim 39 wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium ~~without interfering with subsequent growth of the plant cells~~, said wash carried out for between half an hour to overnight in duration.

46 (amended). A method for pine cell tissue culture which comprises culturing pine cells of the genus *Pinus* subgenus *Pinus* on a support membrane placed over a gel medium.

47 (amended). The method of claim 46, wherein said support membrane is placed over a layer containing one or more tissue culture medium constituents ~~components~~, said layer is positioned on said gel medium.

49 (amended). The method of claim 47, wherein said layer is a ~~thin film~~ layer of liquid medium.

52 (amended). A method for selecting transformed cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells of the *Pinus* subgenus subsequent ~~which have been subjected~~ to transformation on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and

selecting transformed cells.

55 (amended). The method of claim 54, wherein said layer is a ~~thin film~~ layer of liquid medium.

58 (amended). A method for eradicating *Agrobacterium* from cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells of the *Pinus* subgenus on a support membrane over a layer containing an eradicant, said layer positioned in or over a gel medium; and

recovering cells from which said *Agrobacterium* contaminant has been eradicated.

59 (amended). The method of claim 58, wherein said layer is a ~~thin film~~ layer of liquid medium.

60 (amended). The method of claim 58, wherein said layer is a ~~thin film~~ layer of gelled medium.